THE STREPTOCOCCI OF THE ACTINOMYCES-LIKE GRANULES OF THE TONSILS

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In 1914 one[1] of us called attention to the nature of the actinomyces-like masses that are commonly found in the crypts of the faucial tonsils. At that time a series of 122 pairs of tonsils were carefully examined for the presence of these bodies which were found in 30. Now a larger series has been examined and certain new facts have been gathered with reference to the nature of the streptococci found in them.

The granules are grayish or yellowish bodies, rather firm, and on microscopic examination have a raylike structure resembling the sulphur granules of actinomycosis for which they have been mistaken. Their size is variable. They are usually about the size of a pinhead, but not infrequently larger. In one instance, a granule measured 15 mm. in all dimensions. They may be so small as to be visible only with the aid of a hand lens or microscope. In the crypts of the tonsils, they lie free either near the base or nearer the orifice through which they may protrude. They are quite brittle and when disintegrated have a foul and disagreeable odor.

The frequency of their occurrence is difficult to estimate. Careful examination of smears from the crypts of almost all tonsils will reveal such masses in minute form. As stated, in the first series the granules were visible to the naked eye in 30 of 122 pairs of tonsils. In the present series they were found in 31 of 135 pairs. If they are present, there is usually more than one. If single it is often large. Small granules occur in numbers of 3 or 4 or more. In one instance as many as 20 were found. During the process of enucleation of the tonsils many are frequently lost.

Microscopically they have a raylike structure made up of filaments, and at the periphery of brushlike structures which consist of a central shaft of filaments about which are arranged large numbers of bacilli, spirilla and cocci. The bacilli are perpendicular to the central shaft.

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[1] Davis: Jour. Infect. Dis., 1914, 14, p. 144.

They are anaerobic organisms, and resemble in their morphologic and cultural characteristics the various forms of the fusiformis group.

In the present study particular attention is directed to the streptococci associated with the filamentous forms. In smear preparations from the crushed granules, they appear in pairs, less often singly or in short chains. They intermingle freely in variable numbers with the bacilli and spirilla, and do not seem to have any characteristic distribution. In some granules the cocci are very few in number, while in others they are the predominating organisms. They are strongly gram-positive.

For isolation of these cocci the granules were collected immediately after tonsillectomy from patients, chiefly children, in the ages of 5-16. The granule was first washed several times in sterile salt solution and then crushed by a sterile knife. Plain agar with a reaction of +1 to which defibrinated human blood was added in the proportion of 1:20, was inoculated with a loopful of material, and then poured into Petri dishes. Three dilutions were made. The plates were then incubated under both aerobic and anaerobic conditions. To remove the oxygen, an alkaline pyrogallic acid mixture was placed in the bottom of a desiccating jar. The plates were placed above, and the jar was firmly sealed to exclude the air. The anaerobic plates were incubated for 48 hours.

Two kinds of streptococcus colonies would develop in both aerobic and anaerobic plates. There were small colonies surrounded by clear zones of hemolysis measuring in width from 3-5 mm.; the other type was surrounded by a green or grayish-green zone. The proportion in which these colonies occurred, in the aerobic and anaerobic plates, is shown in Table 1. We see that the colonies on the anaerobic plates are generally more numerous than those on the aerobic plates; that the viridans type is constant, while the hemolytic type may or may not be present. Although the number of hemolytic colonies are more numerous on the anaerobic plates, the growths on the aerobic and anaerobic subcultures are equally profuse. In the case of the viridans type, there may be no growth on the aerobic plates and no growth or a very fine growth on the first aerobic subcultures. In the subsequent subcultures the growth becomes gradually heavier until the third or fourth transfers when the growth is equally heavy in the aerobic and anaerobic slants. The anaerobic subcultures were made on blood-agar slants using the Wright alkali-pyrogallic acid method.

Tall tubes of ascitic dextrose agar were inoculated with 22 strains. In all instances both the hemolytic and viridans types preferred the middle portion of the column, with no growth within 1 cm. of the surface, and little or no growth at the bottom of the tube. Those colonies which grew better anaerobically showed a tendency to grow more luxuriantly in the deeper portions of the tubes. In ascitic dextrose broth these organisms produced a granular deposit on the bottom and sides of the tubes. Some caused a slight or moderate, diffuse turbidity of the medium. Smears from the broth cultures of the hemolytic type, revealed medium-sized or long chains, rarely short chains, and short, sometimes medium-sized chains in cultures of the viridans type. The cocci of the strongly anaerobic strains were considerably smaller in size than those of the indifferent strains.

Four strains of strongly anaerobic cocci which showed small forms in smears were tested for filtrability. An anaerobic blood-agar slant was washed with

TABLE 1

SHOWING THE NUMBER AND CHARACTER OF STREPTOCOCCI FOUND IN THE GRANULES

Number	Hemolytic Streptococci		Streptococcus Viridans		Growth on 1st Subculture				Growth on 2nd Subculture				Growth on 3rd Subculture			
	Aerobic	Anaerobic	Aerobic	Anaerobic	Hemolytic		Viridans		Hemolytic		Viridans		Hemolytic		Viridans	
	Number of Colonies	Number of Colonies	Number of Colonies	Number of Colonies	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
1	1	1	1	800	+++	+++	0	+++	—	—	+	+++	—	—	++	+++
2	37	100	0	10	+++	+++	+	+++	—	—	++	+++	—	—	+++	+++
3	2	100	0	100	+++	+++	0	+++	—	—	+	+++	—	—	++	+++
4	30	0	200	200	+++	+++	+	+++	—	—	++	+++	—	—	+++	+++
5	0	0	50	50	—	—	++	+++	—	—	+++	+++	—	—	+++	+++
6	0	0	0	30	—	—	+	+++	—	—	++	+++	—	—	+++	+++
7	0	200	2	200	+++	+++	+	+++	—	—	++	+++	—	—	+++	+++
8	0	0	100	200	—	—	+	+++	—	—	+++	+++	—	—	+++	+++
9	1	10	25	50	+++	+++	+++	+++	—	—	+++	+++	—	—	+++	+++
10	2	100	2	500	+++	+++	++	+++	—	—	+++	+++	—	—	+++	+++
11	0	0	0	15	—	—	+	+++	—	—	++	+++	—	—	+++	+++

sterile salt solution, and the suspension was filtered slowly through a tested Berkefeld filter. Aerobic and anaerobic cultures of the filtrate were sterile in all instances.

Cultures were made directly from crypts in which the granules were found, in order to compare the streptococcal flora on the crypt epithelium with that of the granules. In Table 2 are given the results of cultures from 4 pairs of tonsils. In all instances the number of colonies on the aerobic and anaerobic plates were about the same. The hemolytic colonies were considerably greater than those of the viridans type. In the granules, it will be recalled, the growth on the anaerobic plates was more profuse and the number of the viridans type of streptococcus exceeded the hemolytic.

Fermentation tests were made with the strains isolated from the granules and from the crypts—22 in all. Litmus ascitic carbohydrate broth was employed. Table 3 shows the results.

TABLE 2

SHOWING THE NUMBER AND CHARACTER OF STREPTOCOCCI FOUND ON CRYPT EPITHELIUM

			Number of Colonies	
			Aerobes	Anaerobes
1	Right tonsil	Hemolytic	500	20
		Viridans	10	4
	Left tonsil	Hemolytic	200	50
		Viridans	8	6
2	Right tonsil	Hemolytic	20	20
		Viridans	0	1
	Left tonsil	Hemolytic	30	30
		Viridans	10	10
3	Right tonsil	Hemolytic	50	30
		Viridans	0	0
	Left tonsil	Hemolytic	100	50
		Viridans	0	0
4	Right tonsil	Hemolytic	150	150
		Viridans	100	100
	Left tonsil	Hemolytic	—	—
		Viridans	—	—

All strains fermented lactose and saccharose; none fermented inulin and mannite. According to the classification of Holman,[2] the hemolytic strains which fermented salicin would fall in the Streptococcus pyogenes group, while those that did not, into the anginosus group. Of the strains here examined there are 5 of the former and 6 of the latter. The viridans strains which fermented salicin belong in the Streptococcus mitis group, while those that did not ferment salicin belong in the Streptococcus salivarius group. In this series, of the former there are 4 and of the latter 7.

Ten strains, 4 hemolytic and 6 viridans, were tested for their pathogenicity in rabbits. The hemolytic form of streptococci were injected intravenously in 2 c c doses of a 48-hour ascitic-dextrose-broth culture. There occurred in all a slight loss of weight, and rise of temperature, followed by rapid swelling of several joints. On postmortem the lesions were those of multiple arthritis, and

[2] Jour. Med. Research, 1916, 34, p. 377.

cultures gave organisms similar to those injected. The viridans forms of streptococci were far less virulent. The centrifugalized sediment of 10-15 cc of ascitic dextrose broth was necessary to produce lesions. The lesions were chiefly of the joints and heart, multiple arthritis, endocarditis, pericarditis being the chief ones. The strongly anaerobic strains did not differ from the others in their pathogenic properties.

TABLE 3

FERMENTATION TESTS OF STREPTOCOCCI ISOLATED FROM GRANULES AND FROM CRYPT EPITHELIUM

No.	Origin	Type	Dextrose	Salicin	Mannite	Maltose	Lactose	Inulin	Saccharose	Raffinose
1	Granules	Hemolytic	+	0	0	+	+	0	+	0
2	Granules	Hemolytic	+	0	0	+	+	0	+	0
3	Granules	Hemolytic	+	+	0	+	+	0	+	0
4	Granules	Hemolytic	+	0	0	0	+	0	+	0
5	Granules	Hemolytic	+	0	0	+	+	0	+	0
6	Granules	Hemolytic	+	+	0	+	+	0	+	+
7	Granules	Hemolytic	+	+	0	+	+	0	+	0
8	Crypts	Hemolytic	+	0	0	+	+	0	+	0
9	Crypts	Hemolytic	+	0	0	+	+	0	+	0
10	Crypts	Hemolytic	+	+	0	+	+	0	+	0
11	Crypts	Hemolytic	+	+	0	+	+	0	+	0
12	Granules	Viridans	+	0	0	+	+	0	+	0
13	Granules	Viridans	+	+	0	+	+	0	+	+
14	Granules	Viridans	+	0	0	0	+	0	+	+
15	Granules	Viridans	+	+	0	+	+	0	+	+
16	Granules	Viridans	+	0	0	+	+	0	+	0
17	Granules	Viridans	+	+	0	+	+	0	+	0
18	Granules	Viridans	+	0	0	+	+	0	+	+
19	Granules	Viridans	+	0	0	+	+	0	+	0
20	Granules	Viridans	+	0	0	+	+	0	+	+
21	Granules	Viridans	+	0	0	+	+	0	+	0
22	Crypts	Viridans	+	+	0	+	+	0	+	+

The occurrence of streptococci with strong anaerobic properties has been noted by several investigators.

Von Gottlieb Salus[3] discusses the frequency of obligate anaerobic streptococci particularly in connection with infections of the female pelvic organs. Special reference is made to a group of anaerobic streptococci termed Streptococcus putridus. It is still an open question whether or not this group consists of organisms of a strictly anaerobic type, or is derived and can be classed under the types included under the name of Streptococcus pyogenes. Warren and Herrick[4] in tabulating 134 cases of bacteremia, observed three instances of anaerobic streptococci in cases of pelvic infections. Curtis[5] isolated an anaerobic streptococcus from a fibroid in one instance. Lingelsheim[6] states that occasionally streptococci occur which on first cultivation require anaerobic conditions, but subsequently may become adapted to aerobic conditions.

[3] Centralbl. f. Bakteriol., O, I, 1916, 77, p. 1.
[4] Am. Jour. Med. Sc., 1916, 151, p. 556.
[5] Jour. Infect. Dis., 1916, 19, p. 712.
[6] Handbuch der Path. Mikroorganismen, 1912, 4, p. 453.

Streptococci of the type isolated in the present series have been cultured from other parts of the body, together with bacilli and spirilla, usually of the fusiform type. Kroening[7] isolated an anaerobic streptococcus in symbiosis with a short curved bacillus. The streptococcus was not pathogenic for animals. Dick[8] in two cases noted the occurrence of Streptococcus viridans associated with Bacillus fusiformis, growing better anaerobically than aerobically. Rosenow[9] found strongly anaerobic streptococci together with fusiform bacilli in 2 cases of appendicitis. Koessler and Moody[10] mention the occurrence of a very small streptococcus with gram-negative fusiform bacilli all anaerobic in the bronchial exudate of patients suffering from bronchial asthma.

The occurrence in the tonsillar crypts of streptococci with strong anaerobic properties, associated with the more strictly anaerobic organism, the Bacillus fusiformis, is interesting and may be another instance of the adaptive property of the streptococcus to its environment.

There is no very clear evidence at present of the pathogenicity of these granules in the tonsils but it may be pointed out that here are foci where organisms with potential pathogenic powers are commonly found and apparently protected. The numerous instances of infectious processes now known caused by hemolytic streptococci or in which streptococci and bacilli of the fusiform types are associated make it necessary to carefully consider the possibility of danger lurking in these foci under special conditions.

CONCLUSIONS

Two types of streptococci, the hemolytic and the viridans, were isolated from the actinomyces-like granules of the tonsils.

The viridans type showed strong anaerobic properties. The hemolytic cocci did not.

Otherwise, the streptococci in their cultural and morphologic characteristics and pathogenic properties resemble streptococci from various parts of the mouth and from the crypts of normal and diseased tonsils.

According to the classification of Holman the hemolytic organisms isolated from the granules are of two varieties, Streptococcus pyogenes and Streptococcus anginosus; the viridans types also are of two varieties, Streptococcus mitis and Streptococcus salivarius.

[7] Centralbl. f. Gynäk., 1895, 16, p. 409.
[8] Jour. Infect. Dis., 1913, 12, p. 191.
[9] Jour. Infect. Dis., 1915, 16, p. 240.
[10] Tr. Clin. Soc. Int. Med., 1917, 1, p. 7.